Appendix A: Scenario 1

1.1 DESCRIPTION

This scenario covers the analogue approach for which the read-across hypothesis is based on (bio) transformation to common compound(s). For the REACH information requirement under consideration, the effects obtained in a study conducted with one source substance are used to predict the effects that would be observed in a study with the target substance if it were to be conducted. The same type of effect(s) or absence of effect is predicted. The predicted strength of the effects may be similar or based on worst case.

1.2 ASSESSMENT ELEMENTS FOR SCENARIO 1

The assessment elements (AEs) for this scenario consist of four AEs common to the analogue-approach and five scenario-specific AEs which depend on the mechanistic explanation (Table A1).

Table A1: Assessment elements (AEs) for Scenario 1

AE #	AE TYPE	AE TYPE
AE A.1	Common	Characterisation of source substance
AE A.2	Common	Link of structural similarity and differences with the proposed prediction
AE A.3	Common	Reliability and adequacy of the source study
AE 1.1	Scenario-specific	Formation of common (identical) compound(s)
AE 1.2	Scenario-specific	The biological targets for the common compound(s)
AE 1.3	Scenario-specific	Exposure of the biological target(s) to the common compound(s)
AE 1.4	Scenario-specific	The impact of parent compounds
AE 1.5	Scenario-specific	Formation and impact of non-common compounds
AE A.4	Common	Bias that influences the prediction

AE A.1 CHARACTERISATION OF SOURCE SUBSTANCE

PURPOSE

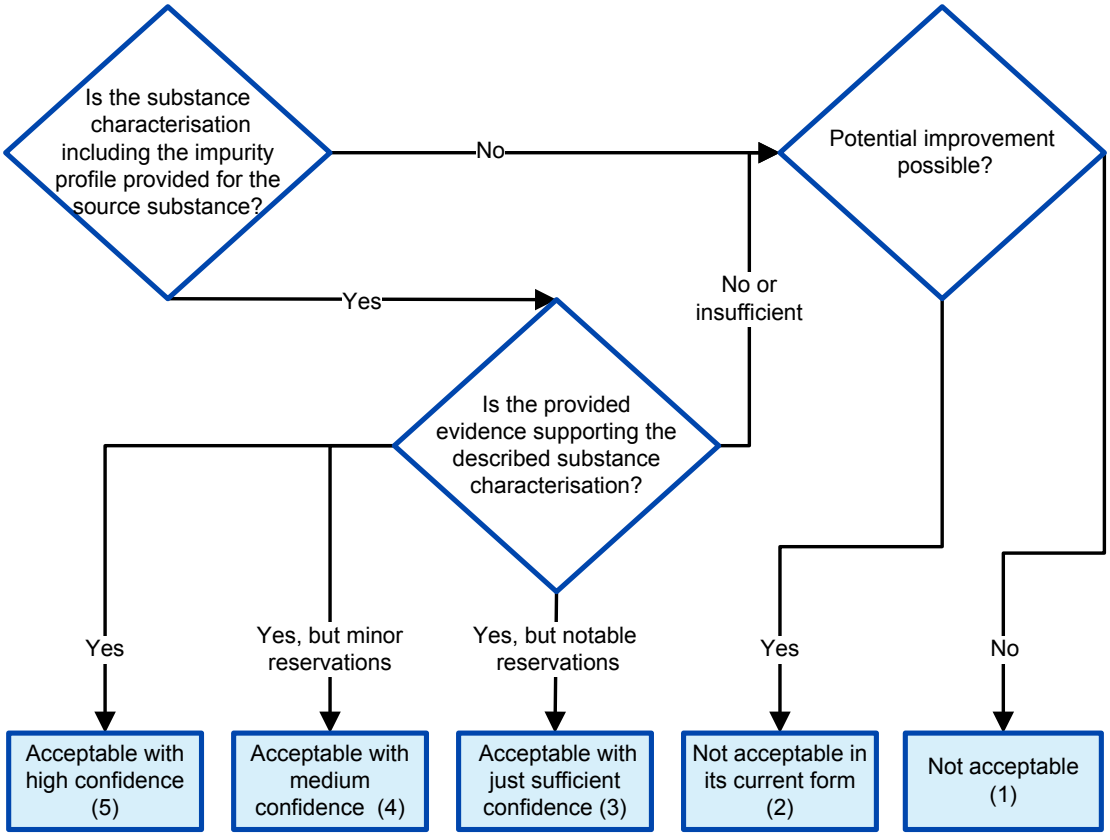
The substance, which is used as the source substance[1], needs to have a clear substance characterisation.

It has to be assessed whether:

- the chemical identity of the analogue is sufficiently clear for a meaningful assessment of the proposed read-across; and
- the impurity profile is clear.

The current AE only looks at the basic information, which allows the comparison of chemical structures to start.

ASSESSMENT OPTIONS



1 The test material actually used in a specific source study is addressed in AE A.3.

EXPLANATION

Structural similarity[2] is a necessary pre-requisite for any prediction based on read-across under REACH. To assess the structural similarity, the chemical identities of the target and source substances have to be clear. This condition is usually met for the target substance, which is registered under REACH, since detailed information has to be provided on the identity, constituents and impurities of the registered substance.

If an adaptation based on read-across is used within an analogue approach, the information provided on the identity of the source substance must establish a clear picture of its chemical structure. It is important that not only the chemical structures but also the impurity profiles of all source and target substances are well defined to establish the read-across hypothesis, since differences in impurities or stereochemistry can affect the activity and chemical properties. It is recommended in the ECHA practical guide "How to report on Read-Across" to follow the Guidance on identification and naming of substances under REACH (version 1.3, February 2014) also for the source substances, not only the substances which are registered.

The source substance should be described as comprehensively as possible and as a minimum[3] the following information should be provided (Guidance R.6.2.6.2):

- Name, CAS and/or EC number, chemical structure for the source substance; and
- Impurities profiles for the source substance (with identifiers as defined above).

Importance of impurities

A mono-constituent substance under REACH is defined by the main constituent, impurities and additives (if appropriate).

Small changes in the impurity profile may have strong effects on toxicological properties. Whilst such may not need to be described to be in compliance with Annex VI (i.e. are allowed in the substance identity description) they may need to be addressed in the hypothesis and justification for a proposed read-across approach.

Read-across has to be based on the structural similarity of the source and target substances. This similarity is based on the main constituents of the source and target substances. However, toxicity may actually be determined by an impurity. The read-across hypothesis could be superficially convincing and could be supported by some data. Nevertheless, the read-across may still be invalid because it does not take a difference in impurity profile of the source and targets substances into account.

The relevance of the impurities for the prediction is assessed in AE 1.4.

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2 Structural similarity alone is not sufficient to justify a prediction based on grouping and read-across. The prediction must be based on the structural similarity, which is to be linked to a scientific explanation of how and why a prediction is possible on the basis of this structural similarity. These aspects are addressed in dependence on the scenario applied by different AEs.
The Board of Appeal stated in the summary of its decision A-006-20132 of 13 February 2014: *" that for a read-across adaptation to be assessed and potentially accepted by the Agency, registrants have to show with clear reasoning and supporting data, set out in the appropriate section of the registration dossier, that the substances involved in the read-across are structurally similar and are likely to have similar properties (or follow a similar pattern). Registrants should also explain how and why the similarity of properties is the result of the structural similarity. The Board of Appeal explained that inclusion of the above information in the dossier is essential to allow the Agency to carry out its role of evaluating whether the read-across proposal complies with the relevant provisions of the REACH Regulation."*

3 Depending on the property under consideration in the read-across approach, the requirements for the substance identity information for the source substance may vary. In some cases, small differences in constituents or impurities may have a strong impact on the toxic properties, even if such differences do not matter in terms of the substance identity information required under REACH.

EXAMPLE(S)[4]

A.1.a Example for an identity of the source substance which is clear and unambiguous and allows a meaningful read-across assessment

- A mono-constituent substance consists of 97.0-99.5% (typical 99.0%) Substance A and 0.5-3.0% identified impurities[5] (typical 1.0% water).

A.1.b Example for an identity of the source substance which is clear and allows a meaningful read-across assessment

- Substance A is a mono-constituent substance.
- The main constituent is present at >70-90% with a typical concentration of 85%.
- The impurity profile[5] is well defined : i.e. Name, CAS and/or EC number, chemical structure and concentration ranges are available for all impurities.

In this case, the identity of the source substance is clear and unambiguous for read-across purposes.

A.1.c Example for an identity of the source substance which is not clear and does not allow a meaningful read-across assessment

- Substance A is a mono-constituent substance
- The main constituent is present at >70-90% with a typical concentration of 85%.
- The impurity profile is not provided

In this case, the identity of the source substance is not clear and is unambiguous for read-across purposes.

4 The examples do not describe a complete set of circumstances. They are to illustrate the specific issues assessed in this AE.

5 The impurity profile of the actual test substance is addressed in A.3; the impact is addressed in AE 1.4.

AE A.2 LINK OF STRUCTURAL SIMILARITY AND DIFFERENCES WITH THE PROPOSED PREDICTION

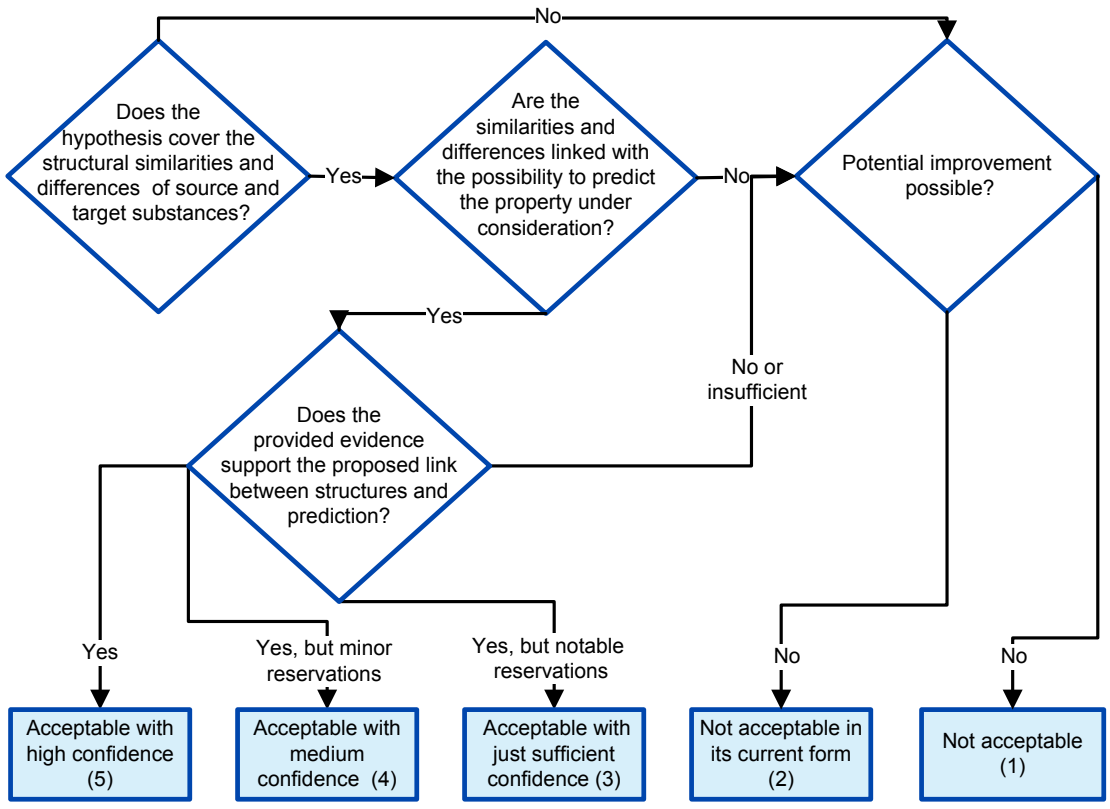
PURPOSE

The aim of this AE is to verify that the source and target substances are covered by the read-across hypothesis.

It has to be assessed whether:

- the scientific hypothesis establishes the structural similarities and differences of the source and target substances;
- structural similarities and differences are linked with the possibility to predict similar properties; and
- the provided evidence supports the proposed link between structural similarities and the possibility to predict.

ASSESSMENT OPTIONS



EXPLANATION

The hypothesis as to why the prediction of similar properties is possible should reflect the structural similarity of the source and target substances.

It should be understood:

1. Which structural moieties or characteristics the source and target substances have in common (for instance, they contain a mono-chloro phenyl moiety or they are primary alcohols of alkanes), and

2. Which structural differences exist (e.g. linear alkyl group may be present at the para position and/or the meta-position of the mono-chloro phenyl ring that contains 1-10 carbon atoms or the chain length of the primary alcohols may vary from C7 to C14).

The explanation should be based on recognition of the structural aspects that the two structures have in common and the differences between the two structures. The possibility for predictions of similar properties should be linked to the common structural aspects.

EXAMPLE(S)[4]

A.2.a Example for a missing consideration of structural differences between source and target substances

- Substance A and substance B are both alpha-olefins;
- Substance A has a linear structure, substance B is branched;
- The hypothesis does not address the branching of substance B.

The explanation also has to address the impact of the branching on the prediction under consideration.

AE A.3 RELIABILITY AND ADEQUACY OF THE SOURCE STUDY

PURPOSE

The source study needs to match the default REACH requirements in terms of reliability and adequacy as requested for any other key study.

It has to be assessed whether:

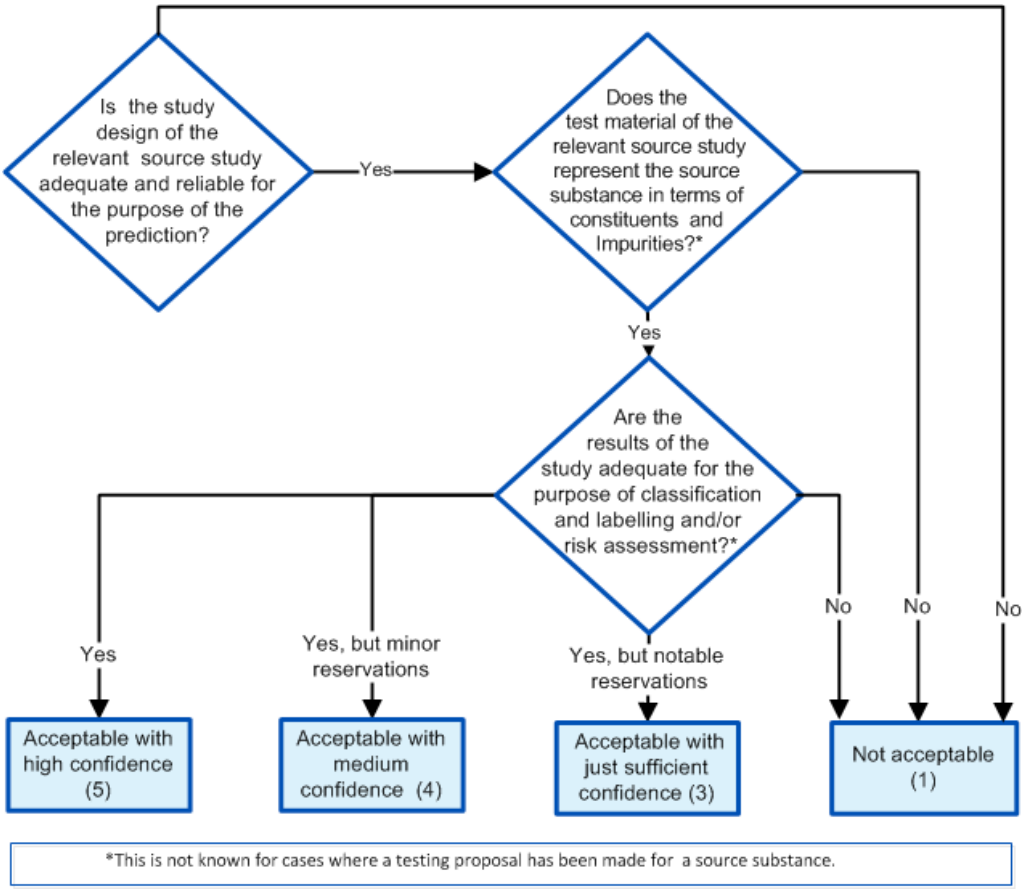
- The study design reported for the source study is adequate and reliable for the purpose of the prediction based on read-across:
    - The study design should cover the key parameters in the corresponding test method referred to in Article 13(3);
    - The study design should cover an exposure duration comparable to or longer than the corresponding test method referred to in Article 13(3); and
    - There is adequate and reliable documentation of the applied test method, i.e. a robust study summary should be provided.
- The test material used represents the source substance as described in the hypothesis in terms of purity and impurities.

It also has to be assessed whether:

- The study results are adequate for the purpose of classification and labelling and/or risk assessment. For example, this could include whether sufficient dose levels have been tested to enable the relevant determination of potency for a decision on classification and labelling, or whether a NOAEL/LOAEL has been identified from a study.

If all conditions listed above are met and the conclusions made are consistent with the reported results (e.g. clear identification of the critical effect(s), reliable NOAEL/LOAEL identification), it may be assumed that the study results are adequate for the purpose of classification and labelling and/or risk assessment.

ASSESSMENT OPTIONS



EXPLANATION

Requirements for source studies

Section 1.5 of Annex XI stipulates that the results of "Grouping of substances and read-across approach" should in all cases:

- *'Be adequate for the purpose of classification and labelling (C&L) and/or risk assessment,*
- *have adequate and reliable coverage of the key parameters addressed in the corresponding test method referred to in Article 13(3),*
- *cover an exposure duration comparable to or longer than the corresponding test method referred to in Article 13(3) if exposure duration is a relevant parameter, and*
- *adequate and reliable documentation of the applied method should be provided.'*

These requirements are placed on the results of the read-across method. Therefore, the source study needs to meet all requirements placed on any key study used as stand-alone evidence to meet an information

requirement under REACH. Therefore, an analysis of the source study used for the prediction of a property needs to be conducted. The elements of the analysis are covered in the purpose section.

Test substance versus source substance characterisation in the hypothesis

There should be no differences in the impurity profile for the test material in comparison with the source substance as covered in the hypothesis. If any such difference is identified, its impact on the prediction should be assessed.

Adequacy for C&L and risk assessment

If the source study is conducted with a test material representative of the source substance, and the study protocol is in accordance with the appropriate international guidelines and good laboratory practice (GLP), sufficient dose levels have been tested to enable the relevant determination of potency for a decision on classification and labelling, and a reliable NOAEL/LOAEL has been identified, the study results may be considered as adequate and reliable and can be used for risk assessment and/or C&L purposes.

If the study has been conducted according to other methods, the deviations need to be evaluated. The Klimisch scores (see below) used by the registrant in the endpoint study record may be helpful for this evaluation, if the assessing expert is able to verify the Klimisch classification of the registrant. A detailed reporting according to the criteria of a robust study summary is needed to assess the characteristics of the source study.

1 = reliable without restrictions: *"studies or data [...] generated according to generally valid and/or internationally accepted testing guidelines (preferably performed according to GLP) or in which the test parameters documented are based on a specific (national) testing guideline [...] or in which all parameters described are closely related/comparable to a guideline method."*

2 = reliable with restrictions: *"studies or data [...] (mostly not performed according to GLP), in which the test parameters documented do not totally comply with the specific testing guideline, but are sufficient to accept the data or in which investigations are described which cannot be subsumed under a testing guideline, but which are nevertheless well documented and scientifically acceptable."*

EXAMPLE(S)[4]

A.3.a Example for a source study not meeting the REACH information requirements

- The source substance was tested in a reproductive toxicity screening test according to OECD 421.
- This study is used to predict the results of a pre-natal developmental toxicity study according to OECD 414 for the target substance to meet the Annex IX requirement of a pre-natal developmental toxicity.

The key parameters of the source study are not appropriate to meet the information requirements of Annex IX, section 8.7.2. The source study is not adequate for the purpose of the intended prediction.

A.3.b Example for a source study conducted with a test substance which significantly differs from the source substance as described in the read-across hypothesis

- The read-across hypothesis refers to a source substance, para-isomer, with a purity of 95%,

impurities are known.

- The structurally similar target substance is also a para-isomer with a purity of 90%, impurities are known.

- A pre-natal developmental toxicity study according to OECD 414 is proposed to be used to predict the pre-natal developmental toxicity study outcome of the target substance. The test material consists of a mixture of para-, meta-, and ortho-isomers of about 35, 20 and 35%, respectively. 10% are unknown impurities.

The test material does not represent the source substance as referred to in the read-across hypothesis.

AE 1.1 FORMATION OF COMMON (IDENTICAL) COMPOUND(S)

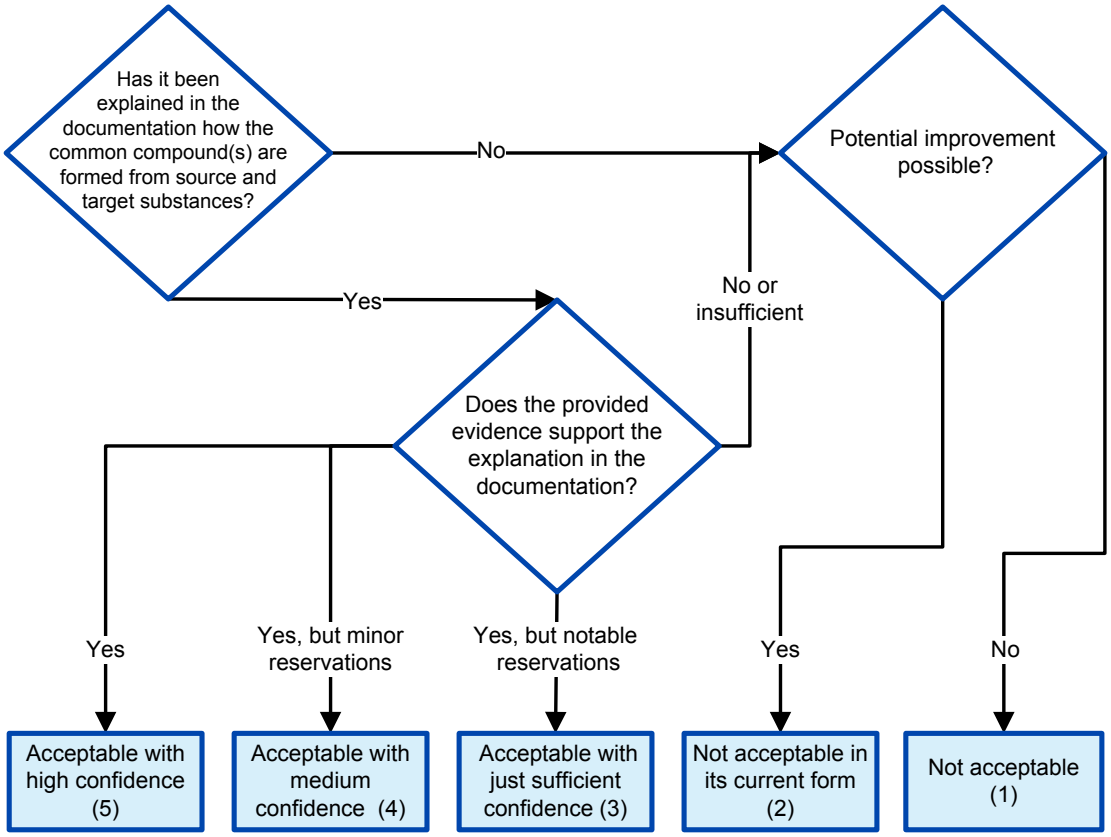
PURPOSE

This AE considers how the common compound(s) are formed from the source and target substance.

It has to be assessed whether:

- it is explained how the (identical) common product(s) are formed (i.e. the product(s) claimed to drive the impact on the property under consideration); and
- the provided evidence supports the explanation.

ASSESSMENT OPTIONS



EXPLANATION

In this scenario, (bio)transformation of source and target substances are claimed to result in local and/or systemic exposure to common (identical) compounds. The prediction of effects or of absence of effect(s) is due to exposure to the common compound(s) only.

This AE is concerned with the formation of the common compound(s), as it is addressed in the scientific explanation. In most cases, the scientific explanation needs the support of case-specific evidence, e.g. experimental data with the source and target substance. However, detailed experimental data such as toxicokinetic studies may not need to be provided. Supporting information may include in vitro, in chemico and in silico studies. The quantitative aspects of the kinetics of the (bio)transformation events are addressed in AE 1.3.

Although this AE is concerned only with the formation of the common compound(s), indirect supporting evidence may be derived also by analysing the data matrix provided by the registrant, i.e. effects in other parameters of multi-parameter studies or effects in studies on other properties. If there is the same clear set of effects in source and target substances, this fact does not contradict that indeed one and the same common compound is formed and is causing the effects. If different compounds would have been formed and cause effects, a regular pattern of effects would be unlikely. Different effects would indicate formation of different compounds or a different rate of formation.

EXAMPLE(S)[4]

1.1.a Example for common compounds

- Substances A and B are converted into the common compound Z.
- Systemic exposure to substance Z is claimed to dominate the effect(s) profile of A and B in the same way.
- Substance A is used as the source substance to predict the property under consideration for target substance B.

The pathway(s) leading from A and B to Z need to be characterised.

1.1.b Example for common compounds

- Source substance A and target substance B.
- A is claimed to be completely and immediately metabolised to B and that the organism is only systemically exposed to B upon external exposure to A.
- Therefore, A and B are claimed to have the same effects.

The pathway[6] leading from A to B need to be characterised.

6 Other (bio)transformation pathways might exist which result in other compounds and are assessed in AE 1.5.

AE 1.2 THE BIOLOGICAL TARGETS FOR THE COMMON COMPOUND(S)

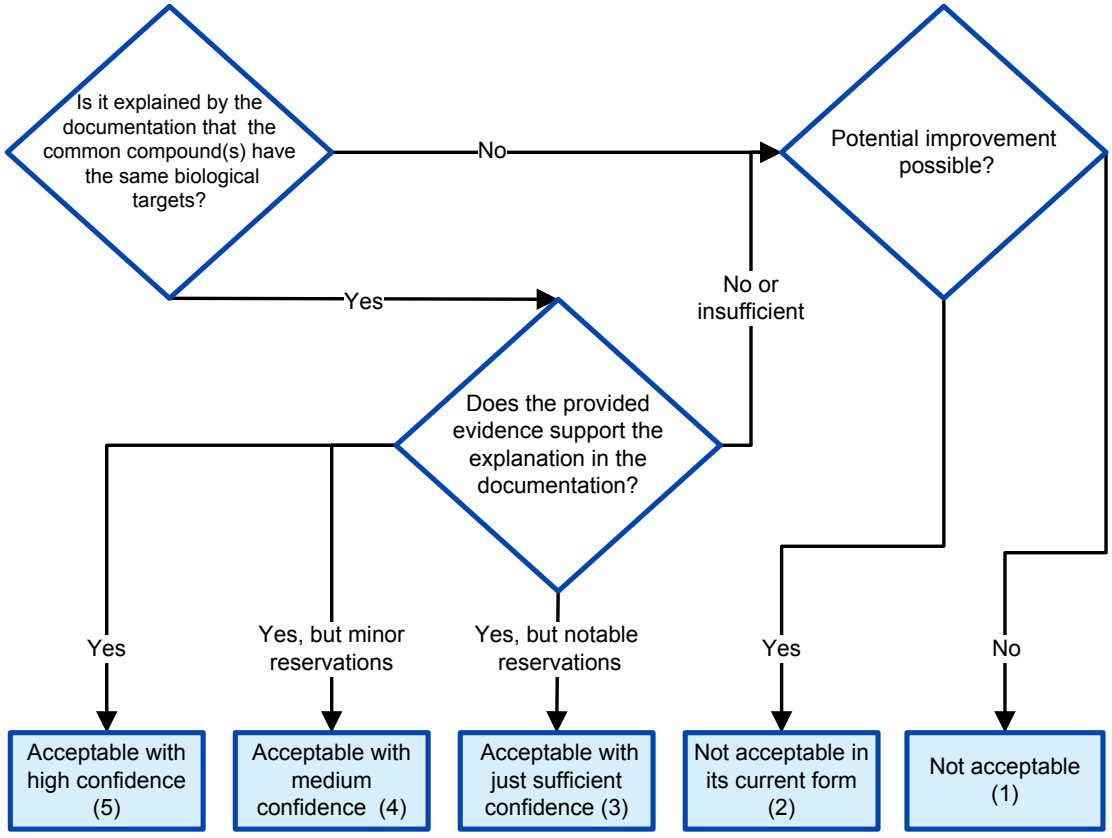
PURPOSE

The hypothesis claims that the common compound(s) have the same biological target(s) (and hence cause the same effects).

It has to be assessed whether:

- the same biological targets are affected by the common compound(s); and
- the provided evidence supports the explanation.

ASSESSMENT OPTIONS



EXPLANATION

The biological targets for the common compounds are supposed to be the same. It must be explained how the (bio)transformation of the source and target substances to the common compounds results in exposure of the same biological targets. It has to be assessed whether the biological targets are indeed the same, e.g. if the same type of effects are observed for the source and target substances.

It is also important to examine the proposed mechanism of formation of the common compound to determine if this provides a reliable basis for prediction of effects in the same target tissue(s) (see AE 1.1)

The fact that common compounds (derived from the source and target substances and assumed to drive the effects) are formed does not always mean that the same biological target is exposed to these compounds. This may be the case when they are formed in different organs and exert their effect directly upon their formation. Another possibility is that due to differences in formation pathways the distribution of the common compounds differ and consequently the exposure of possible target tissues.

Predictions of absence of effects must not be contradicted by the effects profile observed in the data matrix. Note: different effects profiles may also be due to the impact of the parent compounds and/or non-common compounds (see AE 1.4 and 1.5).

The explanations should be supported by evidence.

EXAMPLE(S)[4]

1.2.a Example for occurrence of effects in the same biological targets

- Substances A and B are oxidised to the common compound Z.
- Substances A and B show only nephrotoxicity in 28-day repeated-dose toxicity studies.
- A 90-day repeated-dose toxicity study in A also showed only nephrotoxicity.
- 90-day repeated-dose toxicity study with target substance A is used to predict nephrotoxicity also for B in a 90-day repeated-dose toxicity study.

The supporting evidence (e.g. matrix/mechanistic evidence) does not contradict this prediction.

1.2.b Example for occurrence of effects in different biological targets, although common compounds are formed

- Substances A and B are oxidised to Z.
- A and B are oxidised via different Cytochrome P450s.
- Substance A is largely metabolised to the common compound in the lungs and causes lung toxicity upon oral exposure.
- Substance B is largely metabolised in the liver to the common compound.

Independent of whether A or B are used as the source substance, such circumstances may lead to a rejection of a prediction for the property under consideration.

1.2.c Example for differences in distribution pattern leading to different biological targets for the common compound

- Substance A is converted to substance B in the liver.
- Oral study with B is used to predict the toxicity of A after oral administration.
- Differences in the exposure of organ/tissues to the common compound B have to be expected when exposures are compared between B administered directly or when formed from A.

It has to be explained what such differences in distribution mean for the prediction in terms of biological targets (in addition to kinetic considerations).

AE 1.3 EXPOSURE OF THE BIOLOGICAL TARGET(S) TO THE COMMON COMPOUND(S)

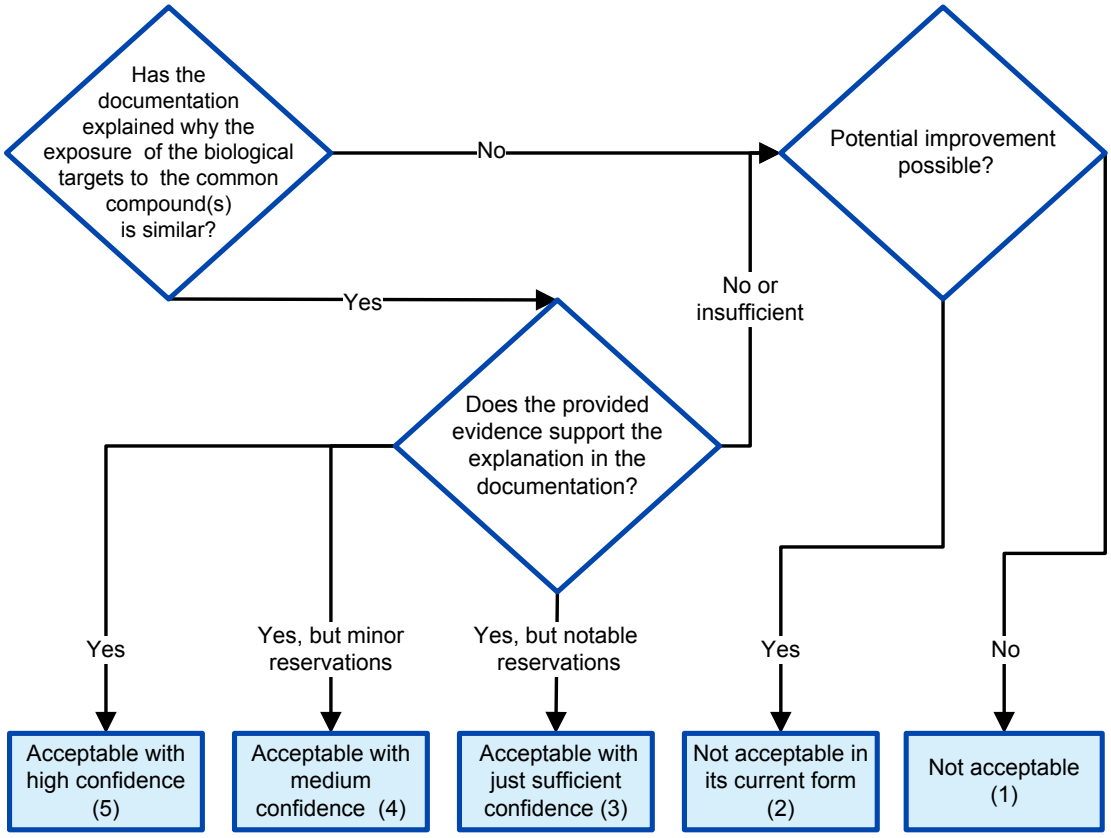
PURPOSE

Under this scenario, it is normally expected that the exposure of the biological targets to the common compound(s) is similar (thereby causing similar strength of effects).

It has to be assessed whether:

- The documentation has explained why the exposure of the biological targets to the common compound(s) is similar; and
- the provided evidence supports the explanation.

ASSESSMENT OPTIONS



EXPLANATION

Mechanistic explanation

Exposure of the biological target(s) to the common compounds derived from the source and target substances may not always be similar. Different blood area under the curve (AUC), peak concentration (Cmax), or time to reach Cmax (tmax) may be indicative of such circumstances. Reasons may be different rates of absorption, metabolism, distribution and/or elimination.

The validity of the read-across depends on the kinetics of the (bio)transformation[7] of the source and target substances to the common compounds. If at equal doses, the kinetics[8] of the (bio)transformation to the common product differs between the source and target substance, the internal exposure pattern will differ. As a result, the hazard of the target substance may be over or under estimated.

Worst case

The read-across justification may claim to use a worst-case approach, i.e. it is not likely to under-predict the effects, but more likely to over-predict. Under this scenario, kinetic aspects leading to higher exposure of the biological target to the common compound in the source substance would be a possible reason for such a claim. It should be noted that the presence of parent compounds and their impact as well as the presence of non-common compounds (see AE 1.4 and 1.5) may invalidate a worst case claim.

Supporting information

The type of information needed to provide sound scientific explanations is case-specific. Quantitative kinetic data are very valuable in this regard. The absence of such information has a serious impact on the robustness of the prediction.

Data matrix

The information presented in the data matrix may also provide indications for differences in exposure, if observed effect levels for the same biological target are significantly different in studies on related properties. However, such differences may also be due to the impact of non-common compounds or the parent compounds (see AE 1.4 and 1.5)

EXAMPLE(S)[4]

1.3.a Example for a difference in absorption resulting in differences in exposure at the biological target

- Substances A and B are (bio)transformed to the common compound Z.
- A has a faster oral absorption rate and higher absorption extent than B.
- As a result, Z has a higher AUC and Cmax in the blood for A than for B (given an equimolar dose of A and B and considering that metabolism rates are similar).
- This difference influences the exposure of the kidneys to the common compound Z thereby also

7 If other (bio)transformation pathways exist (see AE 1.5), there might also be an influence of these pathways (leading to additional non-common compounds) on the kinetics of the pathway leading to the formation of the common compounds.

8 Often only blood kinetics are known. It is assumed that in many cases this can be taken as surrogate for the internal exposure of biological targets. However, this may not be true for all cases.

influencing possible kidney toxicity.

If A is used as the source substance for the prediction of the toxicity of B, it represents a worst case in this example.

If B is used as the source substance, the toxicity of substance A is likely to be under-predicted.

1.3.b Example for a difference in metabolism resulting in differences in exposure at the biological target

- Substances A and B are (bio)transformed to common compound Z.
- A and B have a similar absorption rate, but A has a faster metabolism rate (in the only possible pathway) to Z compared to B (in the only possible pathway).
- As a result, the common product Z has a higher Cmax in the blood after administration of A compared to the Cmax after administration of B (AUCs are assumed to be similar).
- This difference determines the exposure of the bone marrow to the common compound Z thereby influencing the possible toxicity at the bone marrow.

If A is used as the source substance it represents a worst case in this example. If B is used as the source substance, toxicity of substance A is likely to be under-predicted.

AE 1.4 THE IMPACT OF PARENT COMPOUNDS

PURPOSE

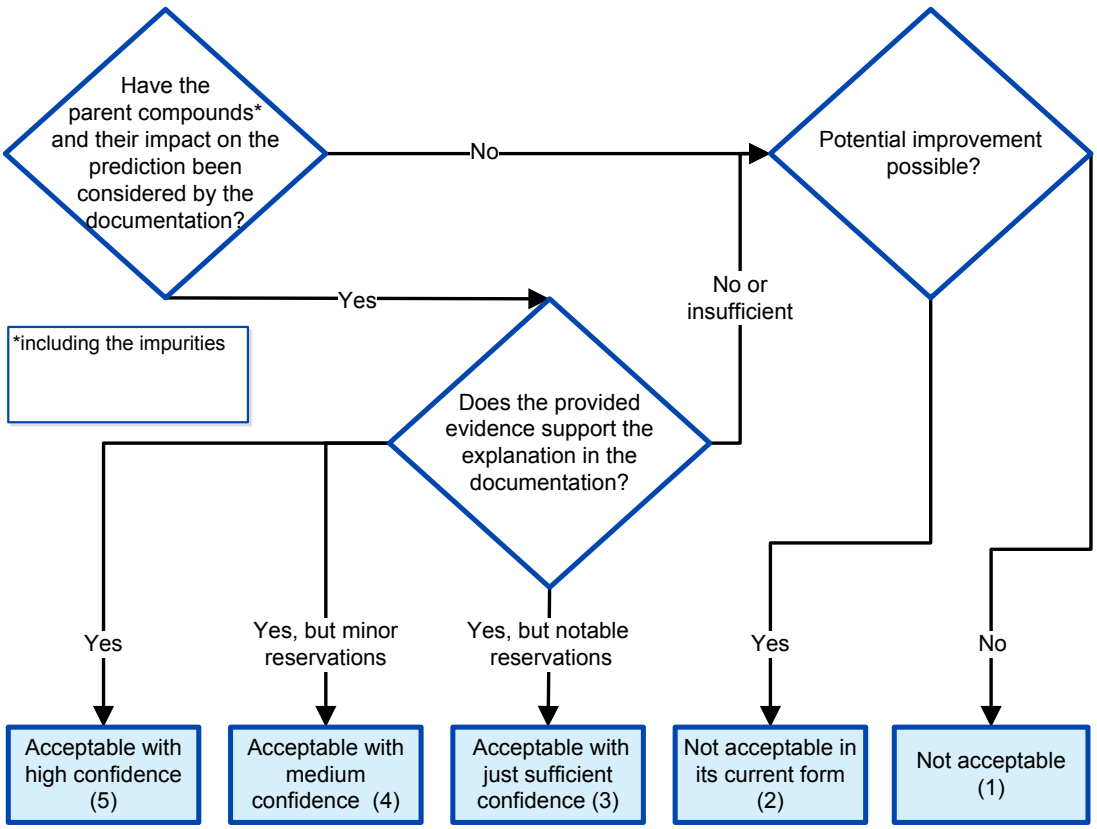
(Bio)transformation of parent compounds may not be immediate and/or complete. As a result, exposure of possible biological targets to the parent compounds may occur for source and/or target substances.

In addition, the impurity profiles[9] associated with the source and target substances may have an impact on the prediction.

It has to be assessed whether:

- the parent compound and its impact on the prediction of the property under consideration has been addressed;
- the identified impurities have an impact on the prediction; and
- the provided evidence supports the explanation.

ASSESSMENT OPTIONS



9 See substance characterisation, as addressed in AE A.1 for the source substance or registration dossier for the target substance.

EXPLANATION

Possible influence of parent compounds

(Bio)transformation of parent compounds may not be immediate and/or complete. As a result, exposure of possible biological targets to the parent compound may occur for source and/or target substances. This AE is related to AE 1.3 where the kinetics of the common compound is covered, which is of course dependent on the(bio)transformation rates of the parent compounds. However, the current AE covers the possible impact of the parent compounds on the predicted effects profile or on the prediction of absence of effects.

Guidance R.6, states that *"in certain instances, the metabolism of the parent compound within barrier tissue (e.g. lung or gut tissue) occurs so rapidly that the initial primary metabolite is the predominant chemical found within the blood. Under these circumstances data from hazard identification studies conducted with that primary metabolite itself can be used to identify hazards for the parent compound."*

If exposure to the parent compound occurs for the source or target substance, it should be explained how this may impact the prediction of the property under consideration. Under this scenario, the parent compound(s) should not significantly influence the predictions. This means that the (bio)transformation of the parent compounds should be rapid and complete or it is known that the parent compound is toxicologically silent. This might prove difficult to establish for higher tier human health endpoints for the target substance.

Supporting evidence

The type of information needed to provide sound scientific explanations is case-specific. Quantitative kinetic data are very valuable in this regard. This is specifically true for predictions of absence of effects.

The information presented in the data matrix may also provide indications for toxicity induced by the parent compounds. If observed effect levels for the same biological target are significantly different in studies on related properties or if different biological targets are observed, such differences may also be due to the impact of the parent compounds. Other possibilities would be the presence of other non-common compounds or kinetic differences.

Importance of impurities

Toxicity of source and target substances may actually be determined by an impurity. The read-across hypothesis could be superficially convincing and could have some supporting data. Nevertheless, the read-across may still be invalid, because it does not take differences in impurity profiles into account.

EXAMPLE(S)[4]

1.4.a Example for barrier tissue

- Substances A and B are converted instantaneously and completely to the only metabolite Z (common compound) in the barrier tissue (e.g. stomach).
- Z is claimed to drive the effects profile, the other (bio)transformation compounds are proven to be biologically inert.
- A repeated-dose toxicity study using the source substance A is used to predict the study results for B.

In this instance, the metabolism of the parent compounds within barrier tissue occurs so rapidly that the parent compound cannot influence the possible toxicity/absence of observable effects.

1.4.b Example for the presence of the parent compound

- Substances A and B are converted to the common compound Z.
- Z is claimed to drive the effects profile.
- The parent compound A is present in significant amounts and for a considerable amount of time, compound B is converted in the liver very rapidly to Z.
- Substance A is suspected to have a toxicity of its own.

If B is used as the source substance, it would only address the toxicity of B and Z but not of A. If Z is used as source substance, prediction for B appears in principle to be possible (however at least exposure of the liver to B will occur which is not addressed by a study with Z).

If A is used as the source substance for B, the toxicity might be dominated by A and not Z, so the hypothesis that toxicity of A and B depends on Z is not correct. In such cases, it has to be assessed whether the impact of the parent compound on the prediction has been covered adequately.

1.4.c Example for a missing explanation in the documentation about the impact of impurities

- Substance A consists of the main constituent A, there is an impurity X of 5%.
- Substance B consists of the main constituent B, there is the same impurity X of 3% and an impurity Y of 2%.
- The documentation only addresses A, B and X.
- Substance A is used as the source substance to predict absence of effect for substance B.
- Y is known to cause nephropathy at low doses.

The documentation also has to address the impurities Y with regard to their possible impact on the prediction.

AE 1.5 FORMATION AND IMPACT OF NON-COMMON COMPOUNDS

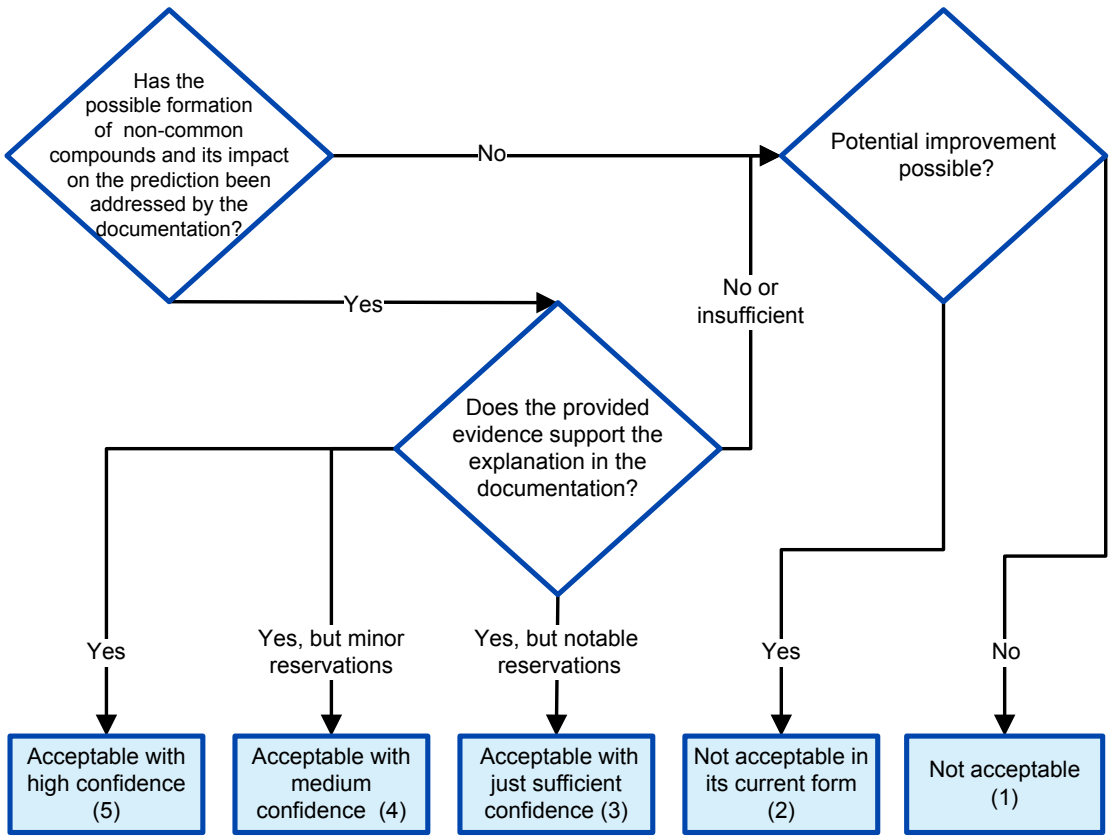
PURPOSE

The formation of common compound(s) often goes together with the formation of non-common compound(s) and possible intermediates which form the common compound(s). Source and/or target substances can also be (bio)transformed via other[10] pathways leading to other additional non-common compounds.

It has to be assessed whether:

- the formation of non-common compounds (including possible intermediates) via the possible pathways and their possible impact on the prediction property under consideration have been considered; and
- the provided evidence supports the validity of the explanation.

ASSESSMENT OPTIONS



10 "Other pathways" are pathways not involved in the formation of the common compound(s) (which are claimed to drive the toxicity).

EXPLANATION

Under this scenario, the prediction for the property under consideration is claimed to be influenced alone by the common (bio)transformation compound(s) of the source and target substances.

Non-common compounds may have the following possible sources[11]:

- formed via the pathway(s) leading to the common compounds;
- formed via (an)other pathway(s); and
- formed as intermediates (all pathways).

As a default, non-common compounds should not impact the prediction of the property under consideration. If a non-common compound is known to have toxicity on its own, it should occur at such high doses that it is not considered to influence the prediction of the property under consideration.

For prediction of absence of effects, the following applies:

- If a non-common compound is formed from the source substance, the results of the source study should show no effects.
- If a non-common compound is derived from the target substance, the absence of effects of this compound should either be obvious or be supported by additional evidence. Otherwise, underestimation of the hazard may occur.

Experimental evidence on kinetics and potential toxicity/absence of effects of the non-common compounds may be necessary for source and target substances.

The analysis of the data matrix provided may provide facts supporting or contradicting the claim. There might be effects on other parameters in multi-parameter studies or effects in studies on other properties. Occurrence of the same clear set of effects in source and target substances does not contradict that indeed one and the same common compound is formed and is causing the effects.

If non-common compounds are formed and cause effects, a regular pattern of effects would be unlikely, if the source and target substances are compared. Different effects would indicate the formation of non-common compounds or influence of parent compounds or different rate of formation for the common compound.

EXAMPLE(S)[4]

1.5.a Example for formation of non-common compounds via the same pathway as leading to the common compounds

- Substances A and B are esters of the same alcohol and two long-chain fatty acids (linear, saturated, C16 and C18).
- After absorption, A and B are rapidly and completely hydrolysed and the test organism is only exposed to the corresponding alcohol and the fatty acids. The fatty acids are the non-common

11 If other pathways which compete with the formation of the common compound exist, there may also be an influence on the kinetics of the formation of the common compounds.

compounds.

- It is explained that toxicity is fully determined by the alcohol.

Evidence for the non-toxicity of the fatty acids should be provided. The possible impact of the parent compounds on the prediction is assessed in AE 1.4.

1.5.b Example for non-common compounds formed via other pathways

- Substance A is converted into common compound Z via one pathway and to A1 via another pathway.
- Substance B is converted into the common compound Z via one pathway and to B1 via another pathway.
- Equal systemic exposure to Z occurs after exposure to A or B which causes a specific histopathological effect in the liver.
- A study conducted with substance A is used to predict the property of substance B.

The formation and possible impact of A1 and B1 on the prediction for the property under consideration should be addressed. The possible impact of the parent compounds on the prediction is assessed in AE 1.4.

AE A.4 BIAS THAT INFLUENCES THE PREDICTION

PURPOSE

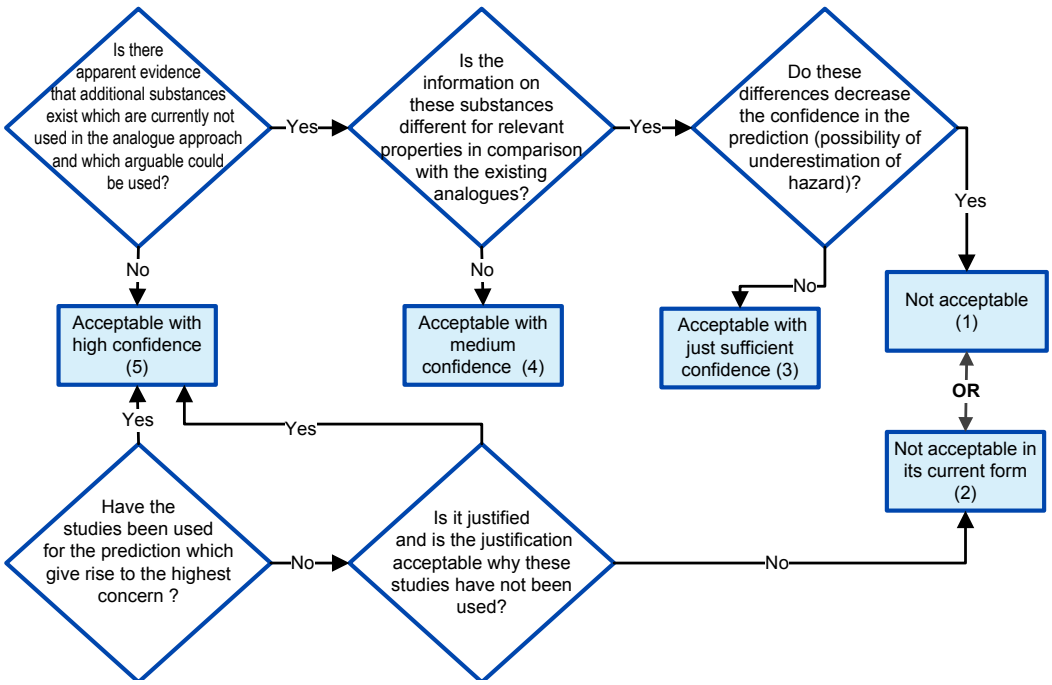
It has to be assessed whether:

- it is clear from the documentation how the source substance(s) have been chosen, for example, what methods/tools have been used to map the field of potential source substance(s), which other substances have been considered and why they have been discarded;
- there are additional, structurally-similar substances which are currently not used in the analogue approach and which arguably could be used;
- there is readily available information from these additional substances;
- this information is biologically significantly different for relevant properties in comparison with the existing analogue(s); and
- these differences decrease the confidence in the prediction (possibility of underestimation of hazard).

It also has to be assessed whether:

- the study(ies) used for the prediction is(are) giving rise to the highest concern for the property under consideration. Justifications have to be provided if the studies giving rise to the highest concern have not been used.

ASSESSMENT OPTIONS



EXPLANATION

There might be information obtained from the dossier or from outside the dossier which triggers concern on selection bias with regard to the source substance(s).

Such a situation may occur:

- when there are multiple possible analogues with equivalent structural similarity; or
- the assessing expert has knowledge of such additional structurally-similar analogue(s).

If the studies conducted with the additional structural analogue(s) have significantly different results for the properties of the substance, then this may result in a difference in the prediction for the property under consideration. Consequently, the proposed prediction may be considered to be unreliable.

In addition there might be selection bias for the study used for the prediction when several studies are available in the data matrix. According to Annex I, section 1.1.4, normally the study giving rise to the highest concern shall be used to establish derived no-effect levels (DNELs). If such a study is not used, this shall be fully justified. This applies to the selection of key studies for predictions based on read-across.